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14. ABSTRACT

The goal was to determine if calcium and vitamin D supplementation can reduce the incidence of stress fracture in female Naval recruits during basic training. The secondary goal was to examine the potential mechanisms for increasing bone adaptation to intense mechanical loading. We recruited 5201 females who were randomly assigned to calcium 2000 mg and vitamin D 800 I.U. per day or a control placebo group. The intervention and stress fracture monitoring continued through 8 weeks of basic training. We were not able to recruit the targeted number of subjects for the sub-study designed to determine changes in moment of inertia using peripheral quantitative computed tomography (pQCT) because the Great Lakes Command directed us to stop the study once we reached our target sample size (5201) for the primary study. For the substudy, we enrolled 148 (out of a target 560). SFx were ascertained when recruits reported to the Great Lakes clinic with symptoms. All SFx were confirmed with radiography or technetium scan according to the usual Navy protocol. A total of 309 subjects were diagnosed with a SFx resulting in an incidence of 5.9% per eight weeks. Using intention-to-treat analysis by including all enrolled subjects, we found that the calcium and vitamin D group had a 20% lower incidence of SFx than the control group (5.3% vs. 6.6%, respectively, P=0.0026 for Fisher's Exact test). The per protocol analysis, including only the 3700 recruits who completed the study, found a 21% lower incidence of fractures in the supplemented vs. the control group (6.8% vs. 8.6%, respectively, P=0.02 for Fisher's Exact test). Generalizing the findings to the population of 14,416 females who entered basic training at the Great Lakes during the 24 months of recruitment, calcium and vitamin D supplementation for the entire cohort would have prevented about 187 persons from fracturing. Such a decrease in SFx would be associated with a significant decrease in morbidity and financial costs.

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INTRODUCTION

Stress fractures during military basic training remain a major concern despite training modifications that have decreased stress fracture incidence. Stress fractures are especially prominent in women. These injuries result in loss of manpower and high medical expense, occasionally incurring service-related disability (1;2;3). Supplementing female recruits with calcium and vitamin D may supply nutrients needed to meet training demands and thereby significantly reduce risk of fracture. Inadequate calcium and vitamin D intake may limit bone adaptation since recruits under 30 years of age have not achieved peak bone mass, training stimulates bone formation and micro-fracture repair, calcium intakes are normally low, and substantial dermal calcium losses occur during training. The goal of this project was to determine if calcium and vitamin D intervention could reduce the incidence of stress fracture in female Naval recruits during basic training. The secondary goal was to examine the potential mechanisms for increasing bone adaptation to intense mechanical loading.

BODY

Key Research Accomplishments

- Implementation of a project that did not interfere with the flow of Naval basic training
- Prompt restart of the project twice, including hiring and training of new study personnel
- Enrollment of 5201 subjects
- Completion of 3700 subjects
- Retention of 71% of enrolled subjects
- Implementation of pQCT sub-study
- Significant findings that calcium and vitamin D supplementation reduce the incidence of stress fracture by 20%.
- Determination of risk factors for stress fracture in female recruits.

Research accomplishments associated with each task outlined in the approved Statement of Work are outlined in Table 1.

Table 1. Research Accomplishments Associated with Tasks in the Statement of Work

	Original	Actual/Projected	Explanation of discrepancies
Planning and set up	7/01-12/01	9/01-5/02	Although the project was funded 9/01, the DOD did not approve us to start until 4/02. We started one month after approval.
Enrollment, intervention and data collection	1/01 - 12/03	5/02 - 4/06	About 4.5 months after startup, enrollment was put on hold by the Great Lakes IRB for nearly 12 months (9/02-8/03). Great Lakes Command directed us to stop recruitment for another 8 months (1/05-8/05).
Data clean up and analysis; manuscript preparation	1/04-6/04	3/06 - 1/08	Delayed as described above

Time Course of Project

After receiving notification of funding, we worked with the DOD regulatory persons and the Creighton and Great Lakes Institutional Review Boards (IRB's) as quickly as possible to obtain approval for starting the study. We did not receive final approval from the DOD until April 2002. We started recruitment one month later. We were actively recruiting until September 26, 2002 when we were notified by the Great Lakes IRB that we were to stop recruitment and put the study on hold. We understand that all active clinical projects were stopped at the Great Lakes at that time. We were allowed to complete follow up of enrolled subjects.

The project was re-approved by the Great Lakes IRB and allowed to resume on August 7, 2003. We needed to hire new staff members since we had no funds to maintain our original staff during the shutdown period. Within five weeks after re-approval we hired and trained new staff, and we resumed recruitment on September 18, 2003. Thus, we were delayed nearly a year with recruitment and data collection.

In September 2003, we were provided money from the DOD to purchase a Stratec peripheral quantitative computed tomography (pQCT) device. We submitted the protocol amendment for the pQCT measurements to the Great Lakes IRB in September 2003 and received approval in January 2004. We immediately requested that the DOD approve the protocol amendment, but we did not receive that approval until June 2004. We then hired and trained a person to do the pQCT measurements and started the pQCT sub-study in July, 2004. We completed the pQCT precision study, and then in October 2004 the pQCT started to malfunction. The first year warranty that was included in the purchase price expired while we were waiting for approval to start the pQCT study. The repair estimate was $10,000. We asked for extra funds from the DOD to repair the pQCT in November 2004. In the meantime, we selected a contractor, obtained a repair quote and put in a purchase order with the university so that we could have the pQCT repaired. In January 2005, the Great Lakes Command directed that we stop all recruitment until we were able to use the pQCT.

We had the device repaired and hired and trained a new technician since our first one had taken another job. We also did a small precision study to assure that the repaired device had reliability. We were ready to restart in June 2005, but the Recruit Training Command at the Great Lakes asked us to wait until August 2005 to restart the study, which we did.

In March 2006, the Command Officers at the Great Lakes ordered us to stop the study because we had reached our target sample size for the main study (5200). We enrolled 5201 subjects in the main study but only 148 in the pQCT sub-study (target sample size – 560).

Assessments

At baseline, participants completed a risk factor questionnaire, and during the study they maintained a record of menstrual periods to ascertain amenorrhea and use of contraceptives. The risk factor assessment was done to determine presence of factors other than properties of bone that are known to contribute to fracture risk: dairy food consumption, previous fractures, family history of osteoporosis, current or past smoking, regular weight-bearing exercise, and use of corticosteroid medication and contraceptives. Dairy food consumption was described as having one or more servings per day of dairy foods. Contraceptive use was defined as having ever used any type of contraceptive (oral, patch, ring, implant, or injection) for more than three months. Weight-bearing exercise was defined as having participated in activity such as walking or running at least three times/week. Subjects who reported smoking were asked to record the number of years they had smoked and the number of packs/day. Similarly, those who reported daily dairy food consumption, regular weight-bearing exercise or use of contraceptives were asked to record the length of time. Lastly, the recruits were asked to estimate the number of alcoholic drinks per week.

Height and weight were self-reported on a subset of the recruits. Body mass index (BMI) was calculated by dividing body weight in kilograms by the square of body height in meters. Fitness at entry to basic training was measured as a 1.5-mile entry run time. During the first week of basic training, each recruit is required to complete a 1.5 mile run in running shoes, and the time to complete that run is recorded. This is done to obtain an estimate of the physical fitness of the recruit. We were able to obtain that value for a subset of the recruits.

Intervention

As individuals consented to the study, they were randomly assigned to the two intervention groups by block randomization. That is, the first two participants enrolled were randomly assigned to one of the two groups, then the next two, and so on. The randomization scheme was known only to the study statistician until the study end. The statistician was responsible for randomization of treatment by subject number and was the only person unblinded.

The study groups were: treatment (2000 mg calcium and 800 IU vitamin D/day) or control (identical placebo). During the eight weeks of training, subjects were asked to take four supplement pills/day made available in the galley line at breakfast and dinner. To monitor pill taking, project staff observed the galley food lines, visited recruits in their quarters, and conducted an exit interview.

Training Conditions

Since recruits within training divisions were randomly assigned to treatment group, both groups were subject to the same training conditions. Thus, even though some training changes occurred during the course of the study, such as a change in boots, subjects in each treatment group experienced any given change in approximately the same numbers. Recruit schedules during basic training are tightly regulated and include one hour of physical training a day, six days a week. In addition, they walked on base to and from classes and the food galleys. During the seventh week of training, recruits' knowledge, skills, strength and endurance are tested during "Battle Stations," in a rigorous training exercise that simulates a combat situation.

The Great Lakes is located at latitude 41° N., and at that latitude very little cutaneous conversion of vitamin D takes place between the months of September and March. Unfortunately, serum 25 hydroxyvitamin D was not measured in our study. We attempted to include that into the study design, but drawing blood was not feasible because of our limited access to the recruits. However, during each season, subjects were randomly assigned to each treatment group.

Stress Fracture Diagnosis

Stress fractures were ascertained when recruits reported to the Great Lakes clinic with symptoms. All stress fractures were confirmed with radiography or technetium scan according to the usual Navy protocol.

Data Analysis

Subjects were characterized by medians and ranges of individual characteristics. Fisher's Exact test and the Cochran-Mantel-Haenszel (C-M-H) test were used to test and estimate differences in fracture incidence between the two treatment groups. For the main intention-to-treat (ITT) analysis, relative risk (RR) and 95% confidence intervals (CIs) were estimated with the C-M-H approach using fracture status (Yes vs. No) as the response variable and treatment group (Calcium+D vs. Placebo) as the factor.

We also performed a number of secondary analyses: (1) per-protocol (PP) with the Fisher Exact and the C-M-H tests; (2) several ITT univariate analyses to determine the effect of individual risk factors; and (3) ITT with a single logistic regression model to determine the effect of categorical covariates while adjusting for treatment, including first-order interaction effects. The odds ratios (ORs) generated by the logistic regression model are good approximations of the respective RRs since fracture is a rare event in our study population (i.e. fracture incidence <10%). The categorical covariates adjusted for in the analyses were: history of exercise sessions per week (High - ≥3 vs. Low - <3), use of depomedroxyprogesterone (Depo) (Yes vs. No), menses during basic training (Yes vs. No), smoking ever (Yes vs. No), age group (Younger - <25 yrs. vs. Older - ≥25 yrs.), and run time per 1.5 miles at entry of basic training (Fast - <15 min. vs. Slow - ≥15 min.).

A Forest plot of the Relative Risk (RR) or Odds Ratios (OR) with 95% CI was generated to demonstrate a) the effect of treatment with Calcium+D from the main analysis; b) the RR of each individual covariate adjusted for treatment; and c) the effect of all covariates adjusted for treatment in a single logistic regression analysis. A level of significance of $P < 0.05$ and the statistical package SAS 9.1 (SAS Inst, Inc, Cary, NC) were used in all analyses.

Results

Subject baseline characteristics are presented in Table 2. There were no significant differences between treatment groups in any of these characteristics. The two progesterone preparations are contraceptives that suppress ovulation and estrogen production by the ovary. During basic training, 2786 subjects reported having no menstrual periods (1391 in the Calcium & D group and 1395 in the Placebo group). The remainder reported having one or more periods. The median dairy intake was less than one serving/day which provides about 300 mg of calcium.

Table 2. Subject Baseline Characteristics				
	Calcium & D Group N = 2626		Placebo Group N = 2575	
Variable	Median (range)	N* (% of group)	Median (range)	N* (% of group)
Age (yrs)	19 (17-35)	2608 (99%)	19 (17-35)	2544 (99%)
Height (in)	64 (52-73)	681 (26%)	64 (49-72)	682 (27%)
Weight (lb)	138 (81-191)	678 (26%)	137 (86-204)	679 (26%)
Body mass index (wt/ht^2)	24 (15-36)	676 (26%)	24 (16-43)	674 (26%)
Entry run time (min/1.5 mile)	16 (11-23)	1575 (60%)	15 (10-23)	1555 (60%)
Dairy servings/wk	6 (1-26)	2543 (97%)	6 (1-26)	2482 (96%)
Alcoholic drinks/wk	3 (1-12)	823 (31%)	3 (1-12)	798 (31%)
Cigarette packyears	3 (1-26)	517 (20%)	3 (1-23)	468 (18%)
Contraceptive pills (yrs use)	2 (1-18)	1143 (44%)	2 (1-17)	1123 (44%)
Depo (yrs of use)	1 (1-11)	424 (16%)	1 (1-13)	403 (16%)
Progesterone implant (yrs use)	1 (1-10)	22 (0.8%)	1 (1-5)	15 (0.6%)
* N= Number of persons for whom data were available or number reporting use; % of recruits in the specific treatment group				

The racial/ethnic distribution of the sample was: American Indian 3%, Asian 4%, non-Hispanic black 18%, Hispanic 13%, non-Hispanic white 54%, and other 4%. The racial/ethnic distribution did not differ between treatment groups.

Of the 5201 subjects enrolled, 365 (7%) were discharged from the Navy before the end of training, and an additional 1136 (21.8%) withdrew from the study. (Figure 1) Therefore, 3700 recruits completed eight weeks of study. Adverse events include any reported symptoms associated with withdrawal from the study. The primary events were gastrointestinal disruption such as constipation, diarrhea, upset stomach (4%) and musculoskeletal soreness (0.9%). Roughly 16% of the subjects quit because they changed their mind, forgot, it was too difficult, or for no reported reason. A small number of subjects withdrew because they were prescribed calcium by Great Lakes medical personnel (1.5%). All enrolled subjects were included in the intention-to-treat analyses. We were able to determine fracture status in subject who withdrew from study but remained on base. We were not able to ascertain fracture status from subjects after they left the military. Subjects who withdrew from study for any reason were excluded from the per protocol analyses.

During basic training, 1843 subjects reported using hormonal contraception. (Table 3) One person reported having a progesterone implant, and she is included among the Depo users in the table.

Table3. Types of Hormonal Contraception Used During Basic Training		
Type of preparation	Calcium & D N* (% of group)	Placebo N (% of group)
Pill, transdermal patch or vaginal ring	649 (25%)	687 (27%)
Depomedroxyprogesterone (Depo) injection	241 (9%)	251 (10%)
Depo injection and pill or transdermal patch	3 (0.1%)	2 (0.07%)
Unknown	6 (0.2%)	4 (0.2%)
None	1727 (66%)	1631 (63%)
N* number of persons reporting; % of recruits randomized to the specific treatment group		

Three hundred and nine subjects (309) were diagnosed with a stress fracture resulting in an incidence of 5.9% per eight weeks. Using intention-to-treat analysis by including all enrolled subjects, we found that the calcium and vitamin D group had a 20% lower incidence of stress fractures than the control group (5.3% vs. 6.6%, respectively, P=0.026 by Fisher's Exact test) with RR = 0.80, 95% CI = 0.64 – 0.99 from the C-M-H test. (Figure 2a) In a per protocol analysis (including only the 3700 recruits who completed the study) there were 21% fewer fractures in the supplemented vs. the control group (6.8% vs 8.6%, respectively, P=0.020 for Fisher's Exact test).

The 309 fracturing individuals sustained 496 fractures. (Table 4.) Most of these fractures were in the tibia or fibula, but 53 were major fractures of the femur or pelvis. The largest relative differences in fracture numbers between treatment and placebo groups were at the tibia/fibula and pelvic sites, but, individually, these differences were not statistically significant. Also, there were no statistically significant differences in fracture incidence among the racial/ethnic groups. The median time to fracture for the entire cohort was 36 days whereas for the placebo group it was 34 days, and for the calcium+D group, 37 days.

Table 4. Fractures by Skeletal Site and Treatment Group		
Skeletal Site	Calcium & D Group	Placebo Group
Tibia/fibula	138	179
Foot	38	34
Pelvis	3	8
Femur	20	22
Other	27	27
Total	226	270

Other risk factors for stress fracture

We performed secondary analyses aimed at determining factors that might increase the risk of fracture in this cohort of female military recruits. The specific factors were selected because they are known to contribute to stress fracture risk. Univariate analyses showed the following factors to be statistically significant predictors of fracture: age, amenorrhea during basic training, entry run time, and history of smoking, regular weight-bearing exercise, and Depo use. A Forest plot is used to demonstrate a) the effect of treatment with Calcium+D from the main analysis; b) the RR of each individual covariate adjusted for treatment; and c) the effect of all covariates adjusted for treatment in a single logistic regression analysis. (Figure 2.)

The risk of fracture in those having amenorrhea was 91% higher than those with one or more menstrual periods during training (RR 1.91, 95% CI = 1.47-2.47, P <0.0001). The risk remained significant when controlled for Depo use. When adjusted for treatment, the risk of fracture in those having amenorrhea was decreased to 83% (P = 0.0035; Figure 2).

In a univariate analysis using age as a continuous variable, every year of age increased the risk of fracture by 5% (RR = 1.05, CI = 1.011-1.090; P = 0.012). Older recruits (Age > 25 yrs) were at a 60% higher risk of fracture than their younger counterparts when treatment was included in the model (P = 0.013; Figure 2b).

The univariate analysis showed that Depo users had a 48% greater risk of fracture than non-users (RR 1.48, 95% CI = 1.18-1.97; P = 0.006). Those who had history of longer use had greater odds for fracture per year of Depo use than those with shorter use (RR = 1.063, 95% CI = 1.01-1.119, P = 0.0188). There was no significant difference in fracture incidence between those who used other contraceptive types and non-users. Adjusting for treatment, those who had a history of Depo use had a 45% higher risk of stress fracture than non-users (P = 0.0057; Figure 2b).

In the univariate analysis, the risk of fracture in recruits with a history of smoking was higher than for those who had not smoked (RR = 1.44, 95% CI = 1.096 – 1.695, P = 0.009). Those who reported a greater number of pack years of smoking had a greater risk than those with fewer pack years (per pack year, RR = 1.044, 95% CI = 1.004 – 1.087; P = 0.03). Adjusting for treatment, recruits with history of smoking had a 41% higher risk of fracture than those who had not smoked (P = 0.0075; Figure 2b).

The median run time of the recruits in this study was 15.5 minutes. The slowest recruit's run time was 23 minutes. In a univariate analysis, the relative risk of fracture was 1.35 for each minute above the median time (95% CI = 1.25-1.45, P = 0.001). Calculations indicate that the risk of fracture in the slowest recruit was almost ten times the risk in the persons with an average run time of 15.5 minutes. When controlled for the effect of treatment, the fast runners (< 15 min per 1.5 miles) had had a 22% smaller risk of incident fracture than the slow runners (P = 0.0373; Figure 2b)

In a univariate sub-analysis within the placebo group, those with a history of high exercise had a 34% lower risk of stress fracture than the low exercise group (5.13% vs. 7.75%, RR = 0.66, 95% CI = 0.49 -0.90; P = 0.008). This exercise history effect was not seen in the supplemented group. Adjusting for treatment, subjects who were in the high exercise (≥3 times/wk) group had a 30% lower risk of stress fracture than those who reported less activity (P = 0.004; Figure 2b)

Dairy food consumption, alcohol use, previous fractures, race/ethnicity, and family history of osteoporosis were not significantly predictive of fracture in this cohort. Also, height, weight and BMI were not associated with fracture risk, but the sample sizes were small since height and weight were not available for all of the subjects.

In a single logistic regression model, we tested the treatment effect as well as the effects of the categorical covariates and their first-order interactions. (Figure 2c) None of the first-order interactions were even marginally statistically significant (P > 0.15); i.e., none of the covariates, such as amenorrhea, interacted with each other. Thus, we do not report them here. In this model, the factors that continued to be significant predictors of increased fracture risk were amenorrhea during basic military training (OR = 1.862, 95% CI = 1.416-2.450, P <0.0001) with 86% increase of incident fracture risk, and age greater than 25 years (OR = 1.658, CI = 1.072) with 66% increase in fracture risk. The only factor that was significantly protective for fracture was history of exercise. Subjects who were in the high exercise (≥3 times/wk) group had a 34% lower risk of stress fracture than those who reported less activity (OR = 0.662, 95% CI = 0.510 – 0.859, P = 0.002).

The rest of the factors in the combined model were marginally significant but pronounced. Thus, Depo users had a 24% higher risk of stress fracture (OR = 1.241, 95% CI = 0.911 – 1.689, P = 0.1713), the fast runners (< 15 min per 1.5 miles) had 20% smaller risk (OR = 0.811, 95% CI = 0.623 – 1.056, P = 0.119), while smokers had a 32% higher risk (OR = 1.319, 95% CI = 0.993-1.751; P = 0.0561), and those on treatment had 20% smaller fracture risk (OR = 0.789, 95% CI = 0.616-1.01, P = 0.0589). Since these effects do not interact, and the odds ratios are good approximations of the respective relative risks, we could use these results to calculate an approximate relative risk of fracture for the worst-case (amenorrhea , age > 25, no exercise, Depo user, slow runner, smoker, no treatment):

$$RR = 1.862 \times 1.658 \times (1/0.662) \times 1.241 \times (1/0.811) \times 1.319 \times (1/0.789) = 11.9$$

This means that the worst-case scenario has 12 times larger fracture risk than the best-case scenario. The relative risk for any other two scenarios can be calculated similarly.

Discussion

To our knowledge, this is the first randomized controlled trial of the efficacy of calcium and vitamin D supplementation that has demonstrated a decreased incidence of stress fracture. Schwellnus et al(4) investigated the effect of calcium supplementation in preventing stress fractures in male military recruits in South Africa but found no statistically significant effect. That study was underpowered since the sample size included only 250 recruits given supplementation, and the calcium supplementation was low (500 mg/day). Only 14 stress fractures were diagnosed over the nine-week training period (incidence of 1.4% in the control group and 0.64% in the supplement group).

It is well established that adequate calcium nutrition is essential for skeletal strength. When serum calcium levels fall due to insufficient intake or excessive loss of calcium, parathyroid hormone (PTH) production is increased in order to stimulate bone resorption and liberate calcium from the skeleton to restore serum calcium concentration. (5;6) This has been called non-targeted (stochastic) remodeling (7;8) and can persist over extended periods of time, as long as the stress to plasma calcium persists. The bone response to the increased PTH is to increase the rate of activation of new remodeling sites, thus increasing the remodeling space. This results in an imbalance of bone remodeling in which more bone is lost from the skeleton than is replaced, and skeletal strength is compromised. Under conditions of intense mechanical loading, such as occurs during basic military training, micro-fractures develop and lead to targeted remodeling to repair the micro-fractures. (7-9) In this situation, optimal levels of circulating calcium are needed to provide substrate for repair of micro-damage and to inhibit an increase of non-targeted remodeling to maintain serum calcium concentration.

High levels of physical activity in the presence of low (or even moderately plentiful) calcium intake can cause additional stress on the skeleton because of the need to offset the substantial cutaneous calcium loss in the sweat. Under conditions of heavy sweating and insufficient calcium intake, calcium is drawn from the bone reservoir under the influence of elevated levels of PTH. In fact, acute bouts of exercise increase PTH levels proportional to exercise intensity.(5;10-13) The cutaneous calcium losses during heavy physical activity can be substantial, (14-16) and the resultant secondary hyperparathyroidism can weaken the skeleton even over short periods. In that regard, Thorsen et al (6) reported that young females showed increased bone turnover, decreased serum ionized calcium and increased serum PTH after a single bout of moderate endurance exercise. This calcium stress may act to limit skeletal adaptation and repair mechanisms in both military recruits and athletes during strenuous activity.

Klesges et al(14) measured cutaneous calcium loss in 11 members of the University of Memphis' men's basketball team during a 10-day training period. Cutaneous calcium loss averaged 422 mg per each two-hour training session. Average total body bone mineral content (BMC) decreased by 3.8% from pre-season to mid-season ($P = 0.02$), a period of four months, while leg bone mass decreased 6% ($P = 0.01$). From pre-season to late summer, the players lost 6.1% of their total BMC and 10.5% of their leg bone mass ($P=0.001$ and $P<0.001$, respectively). Individual players lost as much as 20.2% of their leg bone mass during this 10.5-month interval. During year two of the study, the athletes were supplemented with calcium and vitamin D at doses based on each individual's BMC loss in year one. Supplementation not only stopped losses in BMC, but increased total BMC by almost 2% and leg BMC by 3% by midseason of year two ($P = 0.04$ and 0.05, respectively). This study demonstrates that sweat loss contributes to a considerable loss of BMC in young men who should not be losing bone. Furthermore, sufficient dietary calcium can offset cutaneous losses and allow adequate bone adaptation. The bone loss and subsequent gain with supplementation in the Kleges study were seen over a relatively short period of time, about four months. In our study at the Great Lakes, supplementation prevented stress fracture over about two months.

Although no studies were found using vitamin D supplementation to decrease stress fracture incidence, Ruohola et al (17) found that low baseline 25 hydroxyvitamin D (25[OH]D) predicted stress fracture in Finnish male military recruits. In that study, 800 randomly selected recruits (mean age 19 yrs) were followed prospectively over 90 days, which included eight weeks of basic training. Twenty-two recruits with stress fracture were identified (2.9%). In the final multivariate analysis, a significant risk factor for stress fracture was serum 25OHD below the median level of 75.8 nmol/L (OR = 3.9, 95% CI = 1.2-11.1, $P = 0.002$). It is well established that a small reduction in vitamin D status can contribute to mild increase in parathyroid hormone concentration because vitamin D, in the form of 1,25 dihydroxyvitamin D, regulates the active transport

mechanism of calcium absorption from the gut.(18;19) Bone turnover increases in response to the elevated serum PTH levels. An earlier study of Finnish male recruits, (20) found that high serum PTH levels were associated with stress fracture. These reports support our finding that supplementation with vitamin D can decrease stress fracture incidence.

Supplementation with calcium and vitamin D significantly reduced the risk of stress fracture overall in spite of the negative effects of several lifestyle factors. The supplementation was well-tolerated. In fact, the percent of subjects withdrawing from study due to adverse events was 4% in each of the groups (placebo and treatment). These findings lend confidence that supplementation with calcium and vitamin D is a viable option to for preventing stress fractures, even in populations with factors that significantly increase the risk of fracture.

It is important to note that several factors increase the risk of stress fracture in these female recruits in spite of treatment: amenorrhea during training, age older than 25 years, and history of smoking, low levels of physical activity and Depo use. Also, those who were less physically fit at the beginning of training had a higher risk of fracture than their more fit counterparts.

Poor physical fitness is widely reported to be associated with stress fracture.(20-24) In our earlier study of female Army recruits (25), we found that non-exercisers in the lowest quintile of quantitative ultrasound speed of sound (SOS) had nearly a nine times greater risk of fracture than did non-exercisers in the highest quintile. Thus, strong evidence supports the importance of pre-training activities to improve the fitness of young women gradually before initiating intense physical activity programs. In our Naval study, it is interesting to note that within the placebo group, the high exercise group had a 34% lower risk of stress fracture than the low exercise group, while this exercise history effect was not seen in the supplemented group. Numerous studies of military recruits have found that a history of regular physical activity decreases risk of stress fracture.(21;24-26) Our findings suggest that calcium/D supplementation can somewhat compensate for a history of low physical activity, which is prevalent in U.S. youth, (27;28) the group entering the military services.

Although supplementation lowered the risk somewhat, recruits with a history of smoking still had a 41% higher risk of fracture than those who had never smoked. Other researchers have reported that smoking increases risk of stress fracture.(29-31) Furthermore, numerous investigators have reported an inverse relationship between bone mineral density (BMD) and smoking. (32-36) In our previous study (25), we found that female recruits in the lower quintile of SOS who did not exercise and smoked had a risk of fracture nearly 12 times greater than those with higher SOS values who exercised and did not smoke. Krall and Dawson-Hughes(37) reported that smokers lost bone more rapidly than non-smokers and had significantly lower calcium absorption, suggesting that poor absorption of calcium may contribute to the faster rate of bone loss. In a meta-analysis of the effects of cigarette smoking on bone, Ward and Kleges found that smokers had significantly lower bone mass than non-smokers at all skeletal sites and the difference was dose dependent.(38) Thus, history of smoking in female recruits should serve as a "red flag" indicating high risk of sustaining a stress fracture during basic training.

As we found in an earlier study at a U.S. Army training base,(29) women who had a history of using the long-acting progesterone contraceptive, depomedroxyprogesterone (Depo), had a higher risk of fracture than those who had not used Depo. Similar to other stress fracture studies, (21;24;31) women at the Great Lakes who reported no menses during basic training had a significantly greater risk of stress fracture than those with one or more menstrual periods. This risk remained significant when controlled for Depo use and treatment group. Absence of menses, whether due to Depo use or other causes, is a reflection of very low circulating estrogen levels, which are associated with lower bone mass (39;40) and higher risk of fracture. (41;42)

On average, the Navy recruits in our study had suboptimal calcium intake, 300 mg/day compared to the recommended 1000 mg/day for young women. Thus, it is not surprising that calcium supplementation reduced their risk of stress fracture. As would be expected, our study demonstrates that young women enter basic military training with a variety of risk factors for stress fractures in addition to low calcium intake, such as smoking history, use of progesterone contraceptives, poor physical fitness, etc. The effect of calcium and vitamin D supplementation was strong even when adjusting for these factors. Thus, it seems prudent to provide supplementation to all female recruits.

The incidence of stress fracture in our cohort of young women was 5.9%. This is lower than other reports and may reflect the successful efforts of the Navy to change the skeletal demands of basic training. Our

study shows that supplementation with calcium and vitamin D can provide additional benefits to the successful training changes that have already been implemented by the armed forces.

Although there were only 11 fractures of the pelvis and pubis, there were more than twice as many in the placebo group as in the calcium and vitamin D group. The difference between treatment groups was not statistically significant because of the small numbers of fractures. However, the difference is medically significant considering the morbidity and potential disability, as well as the medical treatment costs, associated with fractures at these sites.

One limitation of this study was that 1136 (21.8%) of the subjects withdrew from the study. However, about 7% of those withdrew so that they could take calcium and vitamin D supplementation provided by Great Lakes medical caregivers and not risk being on placebo. Discounting that group, the attrition is 20%, which is congruent with many clinical trials.

Conclusion

In conclusion, calcium and vitamin D substantially reduced the incidence of stress fractures by 20% in female Naval recruits in this study. Generalizing the findings to the population of 14,416 females who entered basic training at the Great Lakes during the study period, calcium and vitamin D supplementation for the entire cohort would have prevented about 187 persons from fracturing. Such a decrease in stress fracture would be associated with a significant decrease in morbidity and financial costs. Supplementation with calcium and vitamin D provides a safe, easy, and inexpensive intervention that does not interfere with training goals.

Reportable Outcomes

We have made six presentations on this study, and we have been invited to make another presentation at the Stress Fracture Research State of the Science Conference, Columbia SC in February. We have a manuscript accepted for the Journal of Bone and Mineral Research:

Presentations:

Lappe, J., Cullen, D., Recker, R., Thompson, K., Ahlf, R. (2008) Calcium and vitamin D supplementation reduces incidence of stress fractures in Navy recruits. Invited presentation at Stress Fracture Research State of the Science Conference, Columbia SC.

Lappe, J., Cullen, D., Thompson, K., Ahlf, R. (2007) Calcium and vitamin D supplementation reduces incidence of stress fractures in Navy recruits. Meeting of Advanced Technology Applications for Combat Casualty Care, August.

Lappe, J., Cullen, D., Thompson, K., Ahlf, R. (2007) Calcium and vitamin D Supplementation Reduces Incidence of Stress Fractures in Female Navy Recruits. Oral presentation at the Annual Meeting of the Orthopedic Research Society, San Diego CA, Feb 11th.

Lappe, J., Cullen, D., Thompson, K.(2006) Calcium and vitamin D Supplementation Reduces Incidence of Stress Fractures in Female Navy Recruits. Presented to the Command Officers of the Great Lakes Naval Station, June 15th.

Ahlfs, R., Lappe, J., Cullen, D., Thompson, K. (2005) Efficacy of calcium and vitamin D supplementation for the prevention of stress fractures in female Naval recruits. Oral presentation at the Accessions Research & Best Practices Symposium, August 23-25, Lincolnshire IL.

Lappe, J., Cullen, D., Thompson, K.(2004) An update on the study of calcium and vitamin D to prevent stress fractures. Oral presentation to the GlaxoSmithKline Calcium Board. Seattle, September 28.

Lappe, J. (2004) Efficacy of calcium and vitamin D supplementation for the prevention of stress fractures in female naval recruits. Oral presentation. U.S. Army Accessions Command, Accessions Research Consortium, Fort Jackson S.C.

Manuscript

Lappe, J., Cullen, D., Haynatzki, G., Recker, R., Ahlf, R., Thompson, K. (2008) Calcium and vitamin D Supplementation Decreases Incidence of Stress Fractures in Female Navy Recruits. JBMR in press.

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Figure 1. Flow Diagram of Progress through Study Phases. Intention to Treat included all subjects enrolled in the study. Subjects that were discharged or withdrew from study were not included in the Per Protocol analysis.

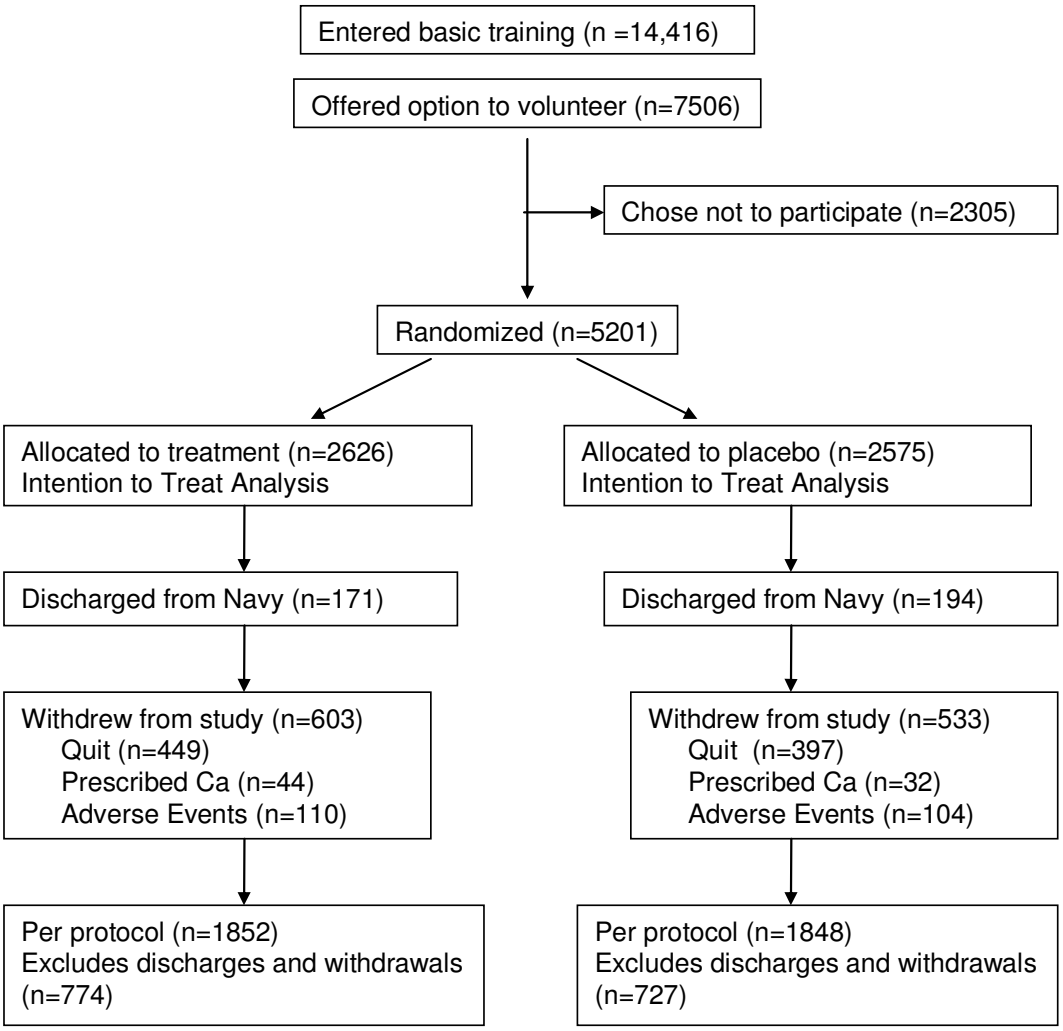

Figure 2. Effect of Treatment Alone, Covariates Adjusted for Treatment, and All Covariates and Treatment Combined in a Single Model. Data represent either Relative Risk or Odds Ratio and the 95% Confidence interval